Free glucocorticoids enter target cells, by still unidentified mechanisms[49, 152] and bind to glucocorticoid receptors causing dissociation of the associated heat shock proteins. Association of hsp90 with glucocorticoid receptors appears to maintain the hormone binding domain in its high affinity conformation.[113] The functional roles of the other associated heat shock proteins are not as well understood, but may include trafficking of the receptor within the cell.[113] The glucocorticoid receptor is a member of a superfamily of transcriptional regulators that include receptors for estrogens, progesterones, androgens, vitamin D, thyroid hormones, and retinoic acid.[83] Members of the superfamily share a similar structure with functional domains for binding of hormone, binding to DNA, and transcriptional activation. Hormone-receptor complex translocates from the cytoplasm to the nucleus. Activated glucocorticoid receptor with hormone bound has an increased affinity for binding to specific DNA sites termed glucocorticoid response elements (GRE) found within glucocorticoid reponsive genes. GREs can either be simple or composite.[48] Most simple GREs consist of two half-site hexamers separated by three nucleotides with resemblance to the consensus sequence GTCACAnnnTGTTCT (SEQ ID NO:1). Association of glucocorticoid receptor, typically as a homodimer, to simple GREs results in enhanced transcription of the target gene. A second type of DNA sequence that binds glucocorticoid receptors, termed composite GREs, has been found in certain glucocorticoid-responsive genes.[31] At composite GREs, the hormone receptor complex interacts with both specific DNA sequences and other transcription factors to regulate transcription.[31, 47, 91] The first demonstrated composite GRE was shown to have binding sites for both the glucocorticoid receptor and activating protein-1 (AP-l).[31] AP-l is a dimer of the oncogene products *c-fos* and *c-jun*. Since glucocorticoid receptors are expressed in many cell types, composite GREs may explain how signal specificity can be achieved in a system with an apparent common final pathway.[48]

**On page 13, delete the 6th full paragraph, and replace this paragraph with the following in accordance with 37 CFR §1.21. A marked up version sh wing changes is attached:**

Figure 6 depicts the genomic structure of recombinant AAV vectors. For reference, the genomic structure of wildtype AAV is shown at the top. Descriptions of each vector can be found in the text. $\beta_2$AR(tag) refers to a cassette that contains the $\beta_2$AR coding region with an epitope (YPYDVPDYA, SEQ ID NO:2) added at the amino terminus of the receptor open reading frame. The epitope tag does not alter $\beta_2$AR function[147] and can be detected with a specific antibody.[100]

**On page 14, delete the 1st full paragraph, and replace this paragraph with the following in accordance with 37 CFR §1.21. A marked up version showing changes is attached:**

Figure 8 depicts putative glucocorticoid response elements (GRE) in the rat $\beta_2$-AR gene. Figure 8A provides a schematic representation of the $\beta_2$AR gene. GREs are number and approximate locations are shown. Figure 8B shows the exact locations (+1 is the start of transcription) of the putative GREs. The third column shows the nucleotide sequence of each GRE (SEQ ID NOS:3-9) compared to the MMTV consensus GRE (SEQ ID NO:10). Underlined nucleotides match consensus. The number of matching mucleotides compared to the consensus GRE are shown in column 4.

**On page 29, delete the 1st full paragraph, and replace this paragraph with the following in accordance with 37 CFR §1.21. A marked up version showing changes is attached:**

Because SPOC1 cells express a wild-type $\beta_2$AR, it is useful to have a method to detect the expression of recombinant $\beta_2$AR in clonal lines infected with recombinant AAV. To accomplish this, an epitope-tagged $\beta_2$AR is used. The cDNA encoding the rat $\beta_2$AR are modified by insertion of the sequence encoding YPYDVPDYA (SEQ ID NOS:11-15) at the amino terminus of the receptor by oligonucleotide-directed mutagenesis. This modification has been performed on the human $\beta_2$AR and has been shown to not alter expression or function of the receptor.[147] This nine amino-acid epitope is recognized by the antibody 12CA5.[100] Thus, immunoblot analysis of membrane fractions prepared from SPOC1 cells can be used to detect recombinant receptor.

Membrane fractions from infected SPOC1 cells are resolved on 10% SDS polyacrylamide gels, transferred to nitrocellulose filters (Schleicher and Schuell, Keene, NH). Immunoblotting is performed in 5% nonfat dry milk containing 2% Nonidet P-40 as previously described using primary antiserum at 1/600 and horseradish peroxidase-conjugated second antibody.[147] The presence of recombinant $\beta_2$AR in clonal cell lines infected with recombinant AAV vector was detected, whereas mock-infected cells did not express the epitope-tagged $\beta_2$AR.

**On page 43, delete the full paragraph which starts on line 12 and ends on page 44, line10, and replace this paragraph with the following in accordance with 37 CFR §1.21. A marked up version showing changes is attached:**

First, corticosteroids are frequently used to treat asthmatic patients. This is done principally to control the inflammatory component of asthma. Therefore, expression of the transgene can be controlled by a therapeutic agent that most asthmatic patients already use. Second, glucocorticoids increase the rate of transcription of several genes including the $\beta_2$AR.[5] This aspect of glucocorticoid action is considered in the design of the optimal $\beta_2$AR transgene for functional testing in airway epithelial cells *in vitro* and *in vivo*. Classically, glucocorticoids exert their effects by binding to a cytoplasmic glucocorticoid receptor causing the release of an associated 90 kDa heat shock protein and thereby allowing translocation of the receptor to the nucleus. Within the nucleus, glucocorticoid receptors form dimers that bind to DNA within steroid-responsive genes at consensus sequences called glucocorticoid response elements. This interaction changes the rate of transcription of the gene, most often resulting in induction of transcription, but in some cases gene expression can be repressed. The present inventors have identified the core GRE in the rat $\beta_2$AR gene as it functions in the HepG2 cell line as discussed below. Based on this work and other evidence, the expression of the rat $\beta_2$AR gene is induced by glucocorticoids. In these studies, the SPOC1 cell line is used to functionally characterize the *cis*-acting elements in the $\beta_2$AR gene that are involved in glucocorticoid induction. Glucocorticoid receptors bind to the consensus sequence GGTACAnnnTGTTCT (SEQ ID NO:10)(where n is any nucleotide). In some instances this may be a straight-forward interaction in which the receptor dimer bound to the GRE then interacts with basal transcription factors[67] or other DNA-binding proteins[126,127] resulting in enhanced transcription of the target gene. However, in many cases the interactions are more complex. At composite GREs, the hormone receptor complex interacts with both specific DNA sequences and other transcription factors to regulate transcription of the target gene.[31,47,91] Some transcription factor binding elements

that interact with glucocorticoid response elements include those for activating protein-1,[31] C/EBP[56] and hepatic nuclear factor 3 (HNF3).[148] Widely spaced glucocorticoid response elements have been shown to function in tandem to induce expression of the tryptophan oxygenase gene.[27] The data obtained from transient expression of $\beta_2$AR-luciferase fusion genes in HepG2 cells indicates complex regulation of $\beta_2$AR gene expression by glucocorticoids that appears to involve other as yet unidentified genetic elements.

**On page 46, delete the full paragraph which starts on line 30 and ends on page 47, line9, and replace this paragraph with the following in accordance with 37 CFR §1.21. A marked up version showing changes is attached:**

To test further the involvement of $GRE_5$ in glucocorticoid regulation of $\beta_2$AR expression, a plasmid p$\beta_2$ARm1(-3129/+126) was constructed that had been mutated at position +6 of $GRE_5$ (GGGTGAGCTGTTCT → GGGTGAGCTATTCT, SEQ ID NOS:16 AND 17). This mutation, the same base change in oligonucleotide m1$GRE_5$ (Figure 10), is essential for glucocorticoid inducibility of a MMTV GRE.[103] The results demonstrate loss of glucocorticoid inducibility using p$\beta_2$ARm1(-3129/+126) (Figure 11). Interestingly, in the absence of added dexamethasone, activity of p$\beta_2$ARm1(-3129/+126) was markedly lower than that of p$\beta_2$AR(-3129/+126) (Figure 11). It appears that basal expression of p$\beta_2$AR(-3129/+126) in HepG2 cells that are over-expressing glucocorticoid receptor is relatively high despite removal of glucocorticoids from serum by charcoal stripping. Forty-eight hours prior to transfection, the HepG2 cells are switched to charcoal-stripped serum. Alternatively, $GRE_5$ contributes to basal activity of the $\beta_2$AR gene promoter.

## REMARKS

Applicant respectfully requests that the foregoing amendments be made prior to examination of the present application.

Applicant believes that the present application is now in condition for allowance. Favorable consideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.